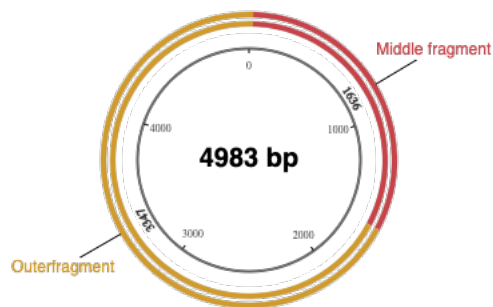# New_Assembly

Created: 3/31/2022, 11:48:19 AM
Saved: not saved

Component Fragments

Name	Length	Produced by	5' End	3' End
Middle fragment	1636	PCR	Fwd Primer (custom)	Rev Primer (custom)
Outerfragment	3391	PCR	Fwd Primer (custom)	Rev Primer (custom)

Notes

- Everything looks OK. No major issues detected.

Required oligos

Name	Primer 5' (overlap/spacer/ANNEAL) 3'	Len	%GC	3' %GC	3' Tm	3' Ta
Middle fragment_fwd	GAGGAACAGAGAGACATTAATGGA	24	42	42	63.3	64.3
Middle fragment_rev	CGCCTGAGCCAGACGAAATA	20	55	55	67.3	64.3
Outerfragment_fwd	TATTTCGTCTGGCTCAGGCG	20	55	55	67.3	64.3
Outerfragment_rev	TCCATTAATGTCTCTCTGTTCCTC	24	42	42	63.3	64.3

Build Settings

Property	Value
Product/Kit	#E5520 NEBuilder HiFi DNA Assembly Cloning Kit
Minimum Overlap	20 nt
Minimum Overlap Tm	48 °C
Circularize	Yes
PCR Polymerase/Kit	Q5 High-Fidelity DNA Polymerase
PCR Primer Conc.	500 nM
Min. Primer Length	18 nt

Assembled Sequence

```
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#DEFINITION  synthetic DNA
#ACCESSION   .
#VERSION     .
#KEYWORDS    NEBuilder
#SOURCE      synthetic DNA construct
#  ORGANISM  synthetic DNA construct
#REFERENCE   1  (bases 1 to 4983)
#  AUTHORS   .
#  TITLE     NEBuilder-generated Construct
#  JOURNAL   Exported  31-MAR-2022 from NEBuilder https://nebuilder.neb.com
#COMMENT     NEBuilder-generated oligos (UPPERCASE = gene-specific, lowercase = overlap)
#COMMENT     Middle fragment_fwd: GAGGAACAGAGAGACATTAATGGA
#COMMENT     Middle fragment_fwd 3'Tm: 63.3   3'Ta: 64.3
#COMMENT     Middle fragment_rev: CGCCTGAGCCAGACGAAATA
#COMMENT     Middle fragment_rev 3'Tm: 67.3   3'Ta: 64.3
#COMMENT     Outerfragment_fwd: TATTTCGTCTGGCTCAGGCG
#COMMENT     Outerfragment_fwd 3'Tm: 67.3   3'Ta: 64.3
#COMMENT     Outerfragment_rev: TCCATTAATGTCTCTCTGTTCCTC
#COMMENT     Outerfragment_rev 3'Tm: 63.3   3'Ta: 64.3
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#                     /mol_type="other DNA"
#                     /plasmid="New_Assembly"
#     gene            1..1636
#                     /note="Middle fragment"
#     gene            1637..23
#                     /note="Outerfragment"
#     primer_bind     1..24
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#     primer_bind     complement(1617..1636)
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#                     /note="gene-specific primer: CGCCTGAGCCAGACGAAATA"
#     primer_bind     1617..1636
#                     /note="Outerfragment_fwd"
#                     /note="gene-specific Tm: 67.3  Ta: 64.3"
#                     /note="gene-specific primer: TATTTCGTCTGGCTCAGGCG"
#     primer_bind     complement(1..24)
#                     /note="Outerfragment_rev"
#                     /note="gene-specific Tm: 63.3  Ta: 64.3"
#                     /note="gene-specific primer: TCCATTAATGTCTCTCTGTTCCTC"
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